Hydration status of lactating women on the Caribbean coast of Guatemala

A cross-sectional study analyzing urinary biomarkers and body composition data

Photo: Kayla Hui

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Abstract

Background: Hypohydration, the condition of body water deficit, can alter bodily functions. Lactating women have extra fluid loss due to breastfeeding. A total fluid intake of 2.7 L/day and 3.8 L/day is recommended according to EFSA and IoM respectively (McKenzie, Muñoz, et al., 2017). In Guatemala, hypohydration during lactation and its consequences are underrecognized and underreported (Rolker et al., 2016; Rush, 2013). Therefore, the hydration status of lactating women from Livingston, Guatemala was studied.

Objective: The aim was to indicate the hydration status of women in any stage of lactation in Livingston, Guatemala by analyzing bioelectrical impedance (BIA) data and morning spot-urine and compare this to a control group. Moreover, four methods determining hydration status were compared.

Design: This cross-sectional study included 80 women, 40 for the Lactating group (LG) and 40 for the Control group (CG). Spot-urine samples were analyzed on color (Ucol), specific gravity (Usg) and osmolality (Uosm) to determine hydration status. Also, BIA was performed for the Lactating group. The data was analyzed with SPSS by comparing the hydration status of LG with CG. Correlation coefficients were calculated for the four methods.

Results: Ucol, Usg and Uosm showed that the majority (Ucol, Usg: 72.5% & Uosm: 50%) of lactating women were hypohydrated, for BIA this was 21%. The control group showed 62.5% (Ucol), 65% (Usg) and 32.5% (Uosm) hypohydration. Only Usg and Uosm were significantly different between the LG and CG. Means for Usg were LG: 1.023 and CG: 1.019 ($p=0.031$), Uosm(log) showed the means LG: 649.53 and CG: 399.02 ($p=0.005$). Ucol, Usg and Uosm were all positively correlated.

Conclusions: Hypohydration is common in lactating women, but also in non-lactating women, on the Caribbean coast of Guatemala. Moreover, Ucol and Usg are inexpensive and easy to use in clinical settings. More research on BIA for establishing hydration status should be done.

List of abbreviations

ANOVA	Analysis of variance
BIA	Bioelectrical Impedance Analysis
CAIMI	Centro de Atención Integral Materno Infantil
CeSSIAM	Center for Studies of Sensory Impairment, Aging and Metabolism
CG	Control group
ECW	Extracellular water
EFSA	European Food Safety Authority
ICW	Intracellular water
IoM	Institute of Medicine
WHO	World Health Organization
LG	Lactating group
SD	Standard Deviation
TBW	Total Body Water
Ucol	Urine color
Uosm	Urine osmolality
Usg	Urine specific gravity
WUR	Wageningen University and Research

Preface

Since the start of my studies on Nutrition and Health, I have had a great interest in mother and child health. The first thousand days of life seem to be very important for further development and health of the child later in life (World Health Organization, 2013). Therefore, the project with the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) on nutrition and health parameters of lactating women got my attention. Hydration is a very important health outcome and being able to study this in different cultural populations in Livingston, Guatemala is of great importance for the community and very interesting. The research project was perfectly in line with my specialization in physiology and health status, since physiological data on hydration status were collected by urine samples and body composition measurements. Furthermore, to be able to work with an international team in a developing country was a great opportunity to learn about planning, conducting and analyzing research in an unfamiliar place with fewer resources. Flexibility, creativity and innovation are important in a research setting in an isolated place like Livingston and I improved these skills during data collection and field work. For data analysis I got a deeper understanding of performing statistical analyses and to critically analyze the data collected and results obtained. Overall, I developed professional skills as a researcher by conducting and analyzing my own data in a country far from my own.

Introduction

Water is an essential nutrient for the human body, without water humans can only survive for a few days. Water takes up about 45-70% of total body mass and it is the major component of body fluids like blood, saliva, synovial fluid and urine, which all perform vital functions in the human body (Gibson-Moore, 2014). Some of the main functions of water are nutrient transport to tissue and waste removal via the hepatic, lymphoid, renal or cardiovascular system. Moreover, water is important for thermoregulation of the body, for example in response to the environmental temperature and physical activity. To maintain total body water, it is important to balance water intake with water losses. If water intake is not sufficient, a person could suffer from dehydration. Dehydration is defined as the process of losing body water and it could lead to hypohydration, the condition of body water deficit (Agostoni et al., 2010; Institute of Medicine, 2005). Dehydration can lead to alterations in bodily functions. It could cause difficulties in concentration, headaches, irritability and sleepiness, as well as it could cause impairments in cognitive function, motor control and cardiovascular function (Agostoni et al., 2010). To prevent people from getting dehydrated, recommendations on water intake are made. According to the World Health Organization (WHO) and the US Institute of Medicine (IoM), adult men and women (19-70+ y/o) are recommended a water intake of 3.7 L/day and 2.7 L/day respectively. This includes water intake from foods and beverages (Grandjean, 2004; Institute of Medicine, 2005). The European Food Safety Authority (EFSA) recommends an intake of respectively 2.5 L/day and 2.0 L/day for adult men and women (Agostoni et al., 2010). Drinking water recommendations are 1.5L of water a day according to EFSA (Agostoni et al., 2010).

For lactating women the recommended daily water intake is higher, since approximately an extra 700 to 850 mL/day of fluid is lost via breastmilk. A total fluid intake of 2.7 L/day and 3.8 L/day is recommended for lactating women according to EFSA and IoM respectively (McKenzie, Muñoz, et al., 2017). Although hydration status of the mother does not seem directly correlated with breast milk osmolality, inadequate hydration status is a direct health risk to the mother, which therefore makes it an indirect health risk for the infant as the mother is the primary caregiver (Rolker, 2016; Soto-Méndez et al., 2016).

In Guatemala, hypohydration during lactation and its consequences are underrecognized and underreported (Rolker et al., 2016; Rush, 2013). Prior studies by CeSSIAM done in the Western Highlands of Guatemala on hydration status and fluid intake of lactating women showed high numbers of inadequate hydration status. A study on maternal hydration status and human milk osmolality showed an 18% higher median in urine osmolality in lactating women than in non-lactating women, which indicates an inferior hydration status (Soto-Méndez et al., 2016). Another study showed that only up to half of the lactating women participating (n=13) were in a state of euhydration (normal state of body water) and an unpublished study on 24-h dietary intake showed that 93% of pregnant and lactating women did not meet the recommended fluid intake by IoM (Rolker et al., 2016). These data show the importance of hydration in maternal and child health and the size of the problem in the Western Highlands of Guatemala.

However, no data is available from Livingston in the region of Izabal, Guatemala. This region is situated on the Caribbean coast and has a tropical climate. The hot and humid climate may cause a greater loss of water due to the physiological response to heat, sweating, and therefore may increase vulnerability to dehydration (Rosinger, 2015b). Moreover, different populations live together in Livingston. The founders of Livingston were the Garifuna, Afro-Caribbean people that are descendants from escaped or shipwrecked slaves and Carib Indians that inhabited the island of San Vicente, the Antilles (Michael Crawford et al., 1981). The Garifuna now live alongside the indigenous Q'eqchi' Mayan that migrated from the region of Alta-Verapaz to Livingston to escape from violence and to find work and land. Livingston is also populated by Ladinos and a small community of Hindu (Aldana de León, 2005). These populations might show differences in eating habits, water intake, body composition and responses to the warm climate, which could result in differences in hydration status. Therefore, this study was done to get insight in the hydration status of lactating women living in the municipality of Livingston, Izabal, Guatemala.

The primary aim of this study was to indicate the hydration status of women in any stage of lactation in Livingston, Izabal, Guatemala by analyzing bioelectrical impedance (BIA) data and morning spot-urine samples for color (Ucol), specific gravity (Usg) and osmolality (Uosm) and to compare this to the hydration status of a control group of non-lactating women. Furthermore, the hydration status of the lactating women was compared for all four methods (Ucol, Usg, Uosm, BIA) separately for cultural origin (Garifuna, Q'eqchi' Maya and Ladina). Also, the hydration status (BIA and Uosm) of the lactating group from Livingston was compared to similar data from the Western Highlands of Guatemala.

The secondary aim was to investigate a correlation between the methods of establishing hydration status, including Ucol, Usg, Uosm and hydration% by BIA. Also, possible correlation between body composition and hydration status, including height, weight, BMI, fat mass and fat free mass was studied.

Expected was that the majority of lactating women on the Caribbean coast of Guatemala would be considered hypohydrated for all four measurements, because of the extra loss of water due to lactation and the tropical climate (McKenzie, Perrier, et al., 2017). Compared to the control group, a significant difference in hydration status was expected between lactating women and non-lactating women for all four methods, with more lactating women being hypohydrated than non-lactating women.

Methods

Data collection took place in the local Health Centre of Livingston, Centro de Atención Integral Materno Infantil (CAIMI), and in the private clinic of Dr. Gladys Contreras, Medical Clinic San Miguel Arcangel. Hydration status of the participants was measured by four different methods, including the urinary biomarkers; urine osmolality, urine specific gravity and urine color. Moreover, body composition data was collected by doing a Bioelectrical Impedance Analysis (BIA).

Study population

In this cross-sectional study the aim was to include a total of 80 women, 40 for the Lactating group (LG) and 40 for the Control group (CG). The Lactating group would include 40 women in any stage of lactation, living in Livingston, region of Izabal on the Caribbean coast of Guatemala. The Control group would include 40 non-lactating women in a reproductive age, meaning an age range from 15 to 50 years old, living in Livingston. Exclusion criteria were women in a non-reproductive age (<15y/o and >50y/o), women with diabetes and women that were diagnosed with gastrointestinal problems on the day of data collection. Also, women taking medication for acute diseases, women taking diuretics habitually and women with episodes of diarrhea the days before data collection were excluded from the study. For the Control group only urine was collected, since a lack of electrodes did not allow to take BIA measurements of the Control group. Participants were selected at the local Health Centre, CAIMI, and at the private health clinic of Dr. Gladys Contreras. All participants signed an informed consent before being included in the study. The aim was to include a similar number of women from Garifuna, Ladino and Q'eqchi' Mayan cultural origin in the study, to be able to investigate differences in hydration status of the different cultural groups. The cultural origin was determined by appearance and wearing or not of traditional clothing. All participants were updated on their hydration status based on urine color and urine specific gravity. They received a small present for their participation.

Measurements

Urinary biomarkers

Urine samples were collected in the period of May 22nd, 2019 to June 7th, 2019. The participants were asked to urinate in a cup in the morning or early afternoon between 8:00h and 14:00h. The urine samples were analyzed on the spot for Ucol and Usg. Moreover, 10 mL was saved and kept frozen in the Medical Clinic San Miguel Arcangel. Uosm was measured in the CeSSIAM office in Quetzaltenango, Guatemala.

Urine color

Urine color (Ucol) is an inexpensive, portable and validated method to establish hydration status. This method is highly correlated with urine osmolality, the standard measure for hydration status (Trinies et al., 2016). The 'Hydration for Health' 8-point chart was used to determine the color of the urine, this chart was developed by Lawrence E. Armstrong and registered with the U.S. Patent and Trademark Office and the European Office for Harmonization in the Internal Market, Trademarks and Designs. The colors 1-3 indicate "well hydrated", 4-6 indicate "not hydrated enough", 7-8 indicate "dehydrated" (Armstrong et al., 1998; "Urine Color Chart - Hydration for Health," 2018). Ucol was determined by two researchers in a well-lit room, placing the urine sample in a clear container on a white background next to the color chart. Both researchers shared their determination, if the determinations did not match, a third researcher would be asked to determine the Ucol and the number of the color that was named twice would be recorded.

Urine specific gravity

Urine specific gravity (Usg) is frequently used as hydration biomarker in clinical settings (Armstrong et al., 2012). Specific gravity refers to the density of a sample compared to pure water. All fluids that are denser than water have a specific gravity higher than 1.000. The range of Usg ranges from 1.000 to 1.040 g/mL with a higher number indicating lower hydration. An accepted cut-off point for dehydration is >1.020 and values above 1.027 are indicated as extremely dehydrated (Armstrong et al., 2012, 2005; Rosinger, 2015b). Similar to Ucol, Usg is an inexpensive, portable and validated method to establish hydration status and highly correlated with urine osmolality (Trinies et al., 2016). A Fisherbrand handheld analog clinical refractometer, model 12-561-341, was used to measure Usg in a well-lit room and the refractometer was calibrated every morning before use. The urine samples were analyzed by two researchers after a few drops of urine were placed on the refractometer, covered and allowed to cool. A third researcher would analyze the specific gravity if the two researchers did not agree on the result.

Urine osmolality

Urine osmolality (Uosm) is a measure of total urine solute content, it measures the number of particles per unit of fluid and is expressed in mOsm/kg (Armstrong, 2005; Middleton et al., 2016). Like Usg, Uosm is frequently used in clinical settings as a biomarker for hydration and is said to be the most accurate urine biomarker available, with the best sensitivity of all urinary biomarkers (Armstrong et al., 2012, 2005; Manz & Wentz, 2003). Uosm was measured using cryoscopic osmometry and performed with a Vogel Löser 815 osmometer. The apparatus was calibrated before use and after each 10 samples by using distilled water, a solution of 300 mOsm/kg and a solution of 900 mOsm/kg. For the measurement, 100 µL homogenized urine sample was used to determine osmolality by the freezing-point depression principle of Peltier (Soto-Méndez et al., 2016). If urine showed higher values than 900 mOsm/kg, the sample would be measured again by using 50 µL of urine and 50 µL of distilled water. The outcome would be multiplied by 2 to get the validated value. Cut-off points for euhydration and hypohydration differ in literature. Armstrong et al. (2016) set a cut-off point for minimal hypohydration at 800 mOsm/kg and Opligger et al. (2005) mentioned 700 mOsm/kg as minimal hypohydration (Armstrong, Johnson, McKenzie, & Muñoz, 2016; Oppliger, Magnes, Popowski, & Gisolfi, 2005). A prior study by CeSSIAM used 900 mOsm/kg as cut-off point for hypohydration (Soto-Méndez et al., 2016). Therefore, it was chosen to use values above 700 mOsm/kg for hypohydration and values above 900 mOsm/kg were used to describe extreme hypohydration.

Bioelectrical Impedance Analysis (BIA)

Besides urinary biomarkers, hydration status was also determined by body composition data obtained through Bioelectrical Impedance Analysis. BIA provides body composition estimates, including total body water (TBW), extracellular water (ECW) and intracellular water (ICW). BIA measures the resistance of an electrical current that flows from hands to feet, which is caused by water and body tissue (Armstrong et al., 2005). The analysis was performed by a SECA mBCA 525 BIA apparatus. Weight, height and waist circumference were measured, and physical activity level was established and recorded in the BIA apparatus. Electrodes were placed on wrists, hands, ankles and feet before the analysis was run. The parameters TBW and ECW were used to calculate the hydration percentage of the participant, with the formula: *Hydration (%) = ECW / (TBW – ECW) * 100* (Software version 1.0 seca 525 Instructions for Use, n.d.).

Euhydration was established for values higher than 73.2%, therefore all values under 73.2% were considered hypohydrated (Bosy-Westphal et al., 2013; Maldonado, Cici Niu, & Solomons, 2018).

In table 1 an overview of the categorization of euhydration, hypohydration and extreme hypohydration was given for each method.

Table 1. Categorization hydration parameters.

	Euhydration	Hypohydration	Extreme hypohydration
Ucol (1-8)[1]	1-3	4-6	7-8
Usg (ml/g)[2]	<1.020	1.020-1.027	>1.027
Uosm (mOsm/kg)[3]	<700	700-900	>900
BIA (hydration%)[4]	>73.2	<73.2	

[1]*(Armstrong et al., 1998; "Urine Color Chart - Hydration for Health," 2018)* [2](Armstrong et al., 2012, 2005; Rosinger, 2015b) [3]*(Armstrong et al., 2016; Soto-Méndez et al., 2016)* [4]*(Bosy-Westphal et al., 2013; Maldonado et al., 2018).*

Statistical analysis

The software SPSS version 26.0 and Excel for Office 365 were used to analyze the data. First, data cleaning was done to detect incomplete and incorrect data. Afterwards a descriptive analysis was performed to present the study population including cultural origin, age, weight, height, waist circumference and BMI. For the Control group only the characteristics cultural origin and age were available, since no BIA was done for these participants. Means and standard deviations (SD) were given for the characteristics age, weight, height, waist circumference and BMI. These characteristics were established for the total population, the Q'eqchi' Mayan, Garifuna and Ladina population separately.

Furthermore, hydration status from all four methods was shown for the total study group, total control group, and separately for cultural origin. A difference between Euhydration, Hypohydration and Extreme hypohydration was made based on the different cut-off points of the four methods (see table 1).

For the associative data analysis part, comparisons between lactating and non-lactating women were made, differences between hydration status per cultural origin were expressed and the four different methods to measure hydration were tested for correlation.

Before running the analyses, data was checked for normality. This was done by a Shapiro-Wilk test, since the sample size is small. Normality of data distribution was checked for all four methods of hydration. If the data was not normally distributed, non-parametric tests or log-transformed data were used. A statistical significance was set at $\alpha < 0.05$.

Independent samples T-tests were used to compare the hydration status of the Lactating group versus the Control group as assessed by Ucol, Usg and Uosm. Also, a comparison was made separately for cultural origin, by comparing outcomes for lactating and non-lactating Garifuna, Q'eqchi' Mayan and Ladina populations. Moreover, a one-way ANOVA test was used to compare the hydration status of lactating women from Garifuna, Q'eqchi' Mayan and Ladino cultural origin. A pair-wise comparison was made using the post-hoc Tukey's HSD test for comparing mean differences in hydration status for all four methods (Ucol, Usg, Uosm, BIA).

A Mann-Whitney U test was used to determine differences in hydration status from BIA and Uosm between the lactating group from Livingston and a dataset of lactating women from the Western Highlands of Guatemala, the region of Quetzaltenango.

To compare the four different methods for determining hydration status, a Spearman's rank-order Correlation test was done. The four methods were also tested on specificity and sensitivity with Uosm as standard.

Finally, correlation tests between body composition and hydration status from lactating women were done with a Spearman's rank-order test. Height, weight, BMI, fat mass and fat free mass were compared with hydration status for the four different methods.

Results

In total 40 lactating women (Lactating Group, LG) and 40 non-lactating women (Control Group, CG) were recruited for the study. Birthdate and age were missing from one participant in the control group, furthermore all data were complete. In table 2, the descriptive data of lactating women from Livingston can be found, with information on cultural origin, age and anthropometrics. The women were in the age range of 18 to 40 years old, with 25 (6) as the mean (SD) age. Included in the study were 24 Q'eqchi' Mayan, 8 Garifuna and 8 Ladina women. The Garifuna women differed significantly in height with the other two cultural origin groups, with Garifuna women being significantly taller (Garifuna: 160.0 ± 3.2 cm vs. Q'eqchi' Mayan: 146.4 ± 6.2 cm and Ladina: 147.9 ± 4.4 cm, $p<0.001$). No other significant differences were found in anthropometrics between the cultural origin groups (Appendix I). The control group included 14 Q'eqchi' Mayan, 10 Garifuna and 16 Ladina women (table 2). The mean (SD) age was 31 (8) years old. For this group, no anthropometric measurements were taken, since BIA data was not collected for this group. Besides the groups from Livingston, a group of lactating women from the Highlands of Guatemala, Quetzaltenango, was compared to lactating women from Livingston. Data for this group was collected in 2017 for another study by CeSSIAM. The descriptive data for this group can also be found in table 2.

The hydration status of the Lactating group and Control group from Livingston are shown in figure 1. Hydration status is shown by the methods Ucol, Usg, Uosm and BIA. According to Ucol and Usg measurements, 72.5% of lactating women were in a state of hypohydration or extreme hypohydration. For Uosm this was 50%, whereas BIA showed 21% of lactating women to be in a state of hypohydration. The control group showed 62.5% and 65% of hypohydration or extreme hypohydration for Ucol and Usg respectively. For Uosm this was 32.5%.

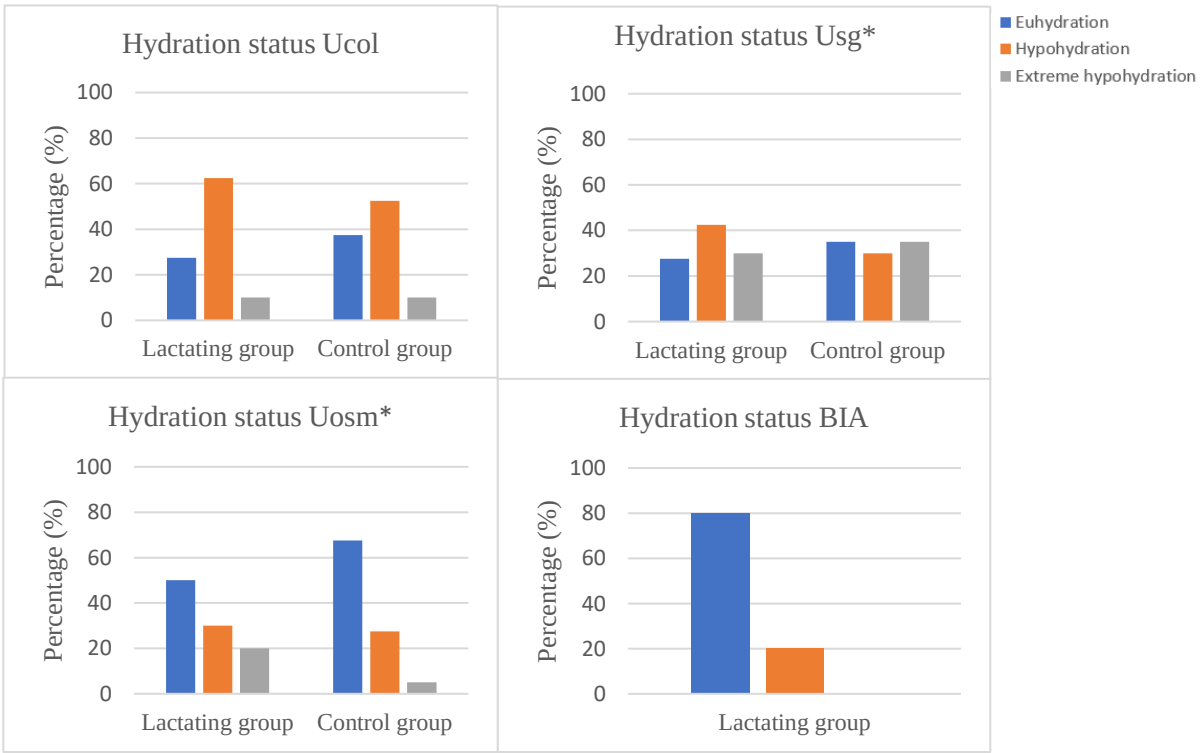

*Figure 1. Hydration status Lactating group vs. Control group for Ucol, Usg, Uosm and BIA. For BIA no control group was available. *Significant difference between LG and CG.*

Figure 2 shows the hydration status of the Lactating group per cultural origin.

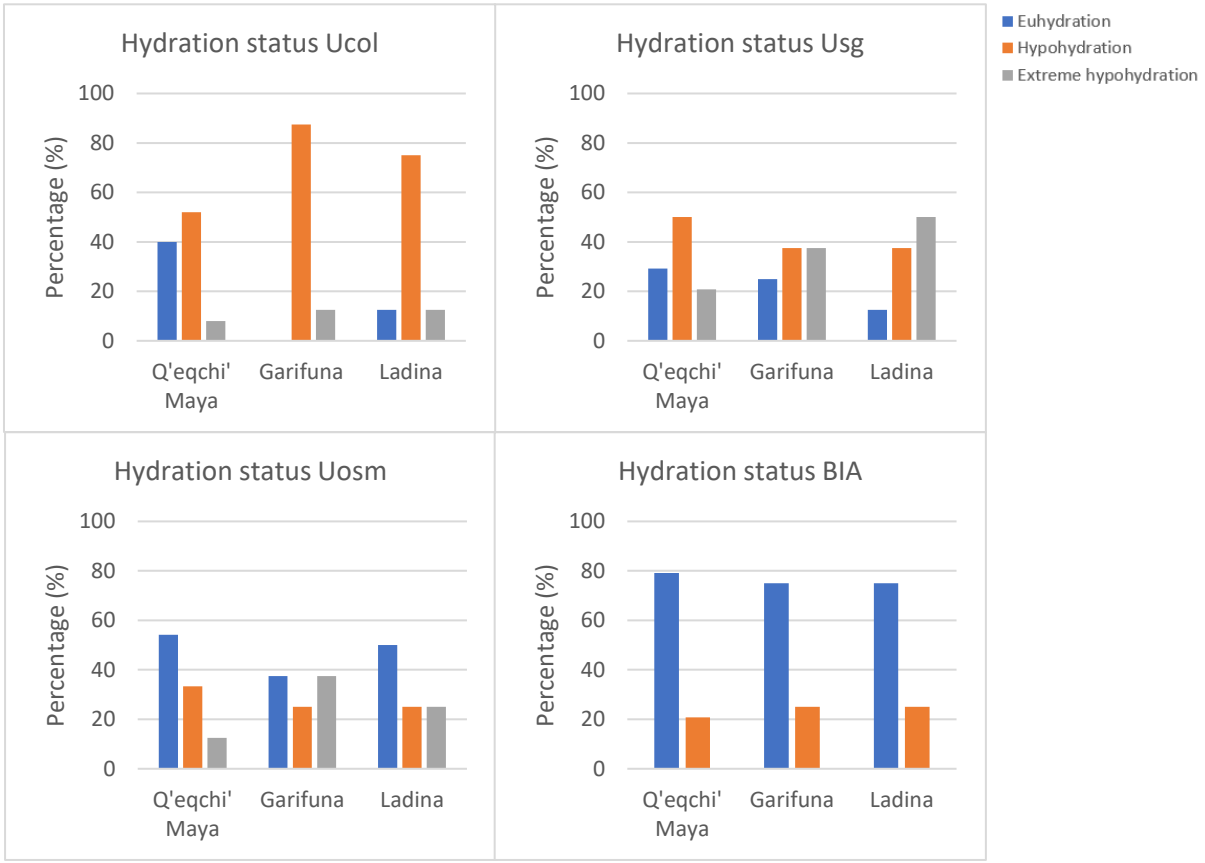

Figure 2. Hydration status Lactating group for Ucol, Usg, Uosm and BIA separately for cultural origin.

Table 2. Descriptive data Study group, Control group and group of lactating women from Quetzaltenango.

	Total LG (n=40)	**Q'eqchi' Maya (n=24)**	**Garifuna (n=8)**	**Ladina (n=8)**	**Total population lactating women Quetzaltenango (n=40)**	**Total CG (n=40)**	**CG Q'eqchi' Maya (n=14)**	**CG Garifuna (n=10)**	**CG Ladina (n=16)**
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ±SD*
Age (years)	25 ± 6	26 ± 7	24 ± 4	25 ± 3	25 ± 5	31 ± 8	32 ± 7	32 ± 8	30 ± 9
Weight (kg)	59.99 ± 15.29	55.65 ± 10.09	67.53 ± 15.19	65.47 ± 22.13	56.98 ± 9.32				
Height (cm)	149.4 ± 7.6	146.4 ± 6.2	160.0 ± 3.2	147.9 ± 4.4	150.9 ± 5.3				
Waist circumference (cm)	83 ± 13	81 ± 9	88 ± 15	87 ± 16	87 ± 9				
BMI (kg/m^2)	26.97 ± 6.48	26.00 ± 4.54	26.97 ± 6.27	29.90 ± 9.86	25.04 ± 3.77				

A Shapiro-Wilk test showed that Ucol and Uosm were not normally distributed, therefore log-transformed data or non-parametric tests were used when needed.

Independent Samples T-tests showed that Usg and Uosm(log) were significantly different between the Lactating and Control group. Means for Usg were LG: 1.023 and CG: 1.019 (p=0.031), Uosm showed LG: 649.53 and CG: 399.02 (p=0.005). This means that lactating women showed an inferior hydration status compared to non-lactating women. Data is shown in table 3.

Table 3. Mean ± SD of hydration markers in the Lactating group and Control group.

	Hydration LG (n=40)	**Hydration CG (n=40)**	**p-value**
Ucol (1-8)	4.29 ± 1.52	4.10 ± 1.53	0.638
Usg (g/mL)	1.023 ± 0.008	1.019 ± 0.008	0.031*
Uosm (mOsm/kg)	649.53 ± 1.96	399.02 ± 2.27	0.005*

*Values are mean ± SD; *significant difference, α<0.05*

Differences in hydration status were also established separately for cultural origin. The non-parametric Mann-Whitney U test was used for Ucol, Usg and Uosm, because Ucol and Uosm were not normally distributed. Q'eqchi' Maya only showed a significant difference between the Lactating and Control group for Uosm (median 679.5 (LG) vs. 413 (CG) (p=0.021)). The Garifuna population showed significant differences for both Usg and Uosm (median Usg: 1.026 (LG) vs. 1.017 (CG) (p=0.026); Uosm: 841.5 (LG) vs. 427.5 (CG) (p=0.013)). In the Ladina population no significant differences were found between the two groups (Appendix II).

Differences in hydration status between the lactating women from Q'eqchi' Maya, Garifuna or Ladino cultural origin were established with a one-way ANOVA. It showed no significant differences between the cultural origin groups for neither of the measurements (Appendix III).

Furthermore, BIA and Uosm data from Livingston were compared with BIA and Uosm data from Quetzaltenango, the Western Highlands of Guatemala. This was done by a Mann-Whitney U test. BIA was not statistically different between groups, whereas Uosm tended towards a significant difference. Medians for BIA were 77.74 (Livingston) vs. 76.30 (Quetzaltenango) (p=0.209), whereas Uosm showed medians of 697.5 (Livingston) vs. 573 (Quetzaltenango) (p=0.089).

A Spearman rank-order correlation test showed that Ucol, Usg and Uosm were all positively correlated. Hydration% was only negatively correlated with Usg (table 4).

Table 4. Correlation matrix of different methods, correlation coefficient (rho) and p-value are shown for every method.

		Ucol	**Usg**	**Uosm**
BIA	*Spearman's rho*	-0.284	-0.418**	-0.311
	p-value	0.076	0.007	0.051
Uosm	*Spearman's rho*	0.544**	0.790**	x
	p-value	0.000	0.000	x
Usg	*Spearman's rho*	0.675**	x	
	p-value	0.000	x	

** *correlation is significant, P<0.01 (2-tailed)*

Moreover, sensitivity and specificity were calculated with Uosm as the standard reference. Usg and Ucol showed higher specificity than sensitivity, whereas BIA showed a better sensitivity than specificity. Outcomes are shown in table 5.

Table 5. Sensitivity and Specificity of Ucol, Usg and BIA with Uosm as standard

	Hypohydration & Extreme hypohydration		
	Uosm >700		
		Sensitivity	*Specificity*
Ucol	***>4***	0.59	0.72
Usg	***>1.027***	0.68	1.00
BIA	***<73.2%***	0.75	0.56

Lastly, correlation between hydration status and body composition measurements were determined. It showed that hydration status (all four methods) was not correlated with height and weight. However, hydration status from BIA was positively correlated with BMI (r=0.437, p=0.004) and with Fat mass (r=0.361, p=0.02). Furthermore, hydration derived from BIA was not correlated with Fat Free Mass (r=0.065, p=0.687) and Visceral fat (r=0.065, p=0.686). Uosm, Ucol and Usg were not correlated with any of the body composition outcomes (BMI, FM, FFM and Visceral fat). TBW and ECW showed significant correlation with height and weight (Appendix IV).

Discussion

The primary aim of this study was to indicate the hydration status of lactating women in Livingston. The data collected showed that hypohydration is common in this region. Two urinary biomarkers (Usg and Uosm) showed that significantly more lactating women than non-lactating women were hypohydrated or extremely hypohydrated. This could be due to the extra loss of water during lactation and was in accordance with the hypothesis (McKenzie, Muñoz, et al., 2017). This pattern was seen in all cultural origin groups, however the Ladina women did not show significant differences between lactating and non-lactating women.

Comparing these findings to prior studies, there is resemblance. A previous study done by CeSSIAM on hydration status in lactating women from the Western Highlands of Guatemala also showed that hypohydration is common, with only half of the women showing a state of euhydration on separate occasions (Rolker et al., 2016). Another study in pregnant and lactating women from Mexico showed that 54% of the lactating women did not reach the recommended daily water intake in the first semester (Martinez, 2014). Moreover, Rosinger (2015) studied the hydration status of lactating women in the Bolivian Amazon. Dehydration was common, with 78% of lactating women and 50% of non-lactating women being dehydrated (Usg >1.020 g/mL). It showed that lactating women had a significantly higher Usg (mean= 1.024 g/mL, p=0.01) and were significantly more dehydrated than non-lactating women. The study suggested that meeting the high water requirements for lactating women is challenging because of the environment they live in. It was stated that, in a population in the Bolivian Amazon, ambient temperature was significantly associated with Usg (Pearson r = 0.66; p = 0.000) (Rosinger, 2015b). The hot-humid environment, little access to clean water and a high burden of infectious diseases increases the risk of dehydration (Rosinger, 2015b, 2015a). Household water insecurity was associated with a 9-fold increased odds of diarrhea for adults and was strongly related to children's odds of dehydration (Rosinger, 2015b). Livingston has a similar environment with a hot and humid climate with an average annual temperature of 26.3°C and clean water access is not guaranteed (Merkel, 2012a). The tropical climate in Livingston could cause a greater loss of water in the people, due to bodily response to hot weather, sweating (Rosinger, 2015b). Therefore, it was expected that hydration status would differ between Livingston and the colder Quetzaltenango, which has an average annual temperature of 13.7°C (Merkel, 2012b). Uosm did show a tendency to a significant difference in hydration status between the two locations, with more dehydration in Livingston.

The secondary aim of the study was to compare four different methods to measure hydration status. Since there is no universally accepted technique to determine hydration status, four methods were considered (Armstrong et al., 2005). The methods were expected to be correlated with one another. However, only Usg, Ucol and Uosm were significantly correlated, which supports literature that these methods are valid in establishing hydration status and can be used in clinical settings (Armstrong et al., 2012, 2005; Kavouras et al., 2016). Ucol was investigated by McKenzie et al. (2017) in pregnant women, lactating women and a control group. It was shown that 24-h Ucol was significantly correlated with 24-h Uosm ($r=0.61-0.84$, $p<0.0001$) and 24-h Usg ($r=0.62-0.90$, $p<0.0001$). Moreover, based on a ROC curve analysis, Ucol of single urine samples was shown to be a useful diagnostic tool for Uosm ≥500 mOsm/kg (AUC=0.909–0.922, $p<0.0001$). The study, however, used different categories for Ucol and Uosm as compared to the current study, with Ucol of 4 indicating a Uosm ≥500 (McKenzie, Muñoz, et al., 2017). Furthermore, a study of Opligger et al. (2005) investigated the validity of Uosm and Usg to measure hydration status and compared this with plasma osmolality. The intervention was done in male athletes and it included acute hypertonic weight loss by dehydration. It stated that Usg and Uosm mimic each other and concluded that Usg and Uosm offer viable methods

in assessing hydration status (Oppliger et al., 2005). Baron et al. (2015) stated that Usg and Uosm are especially suitable to assess hydration status in studies with large sample sizes, since both methods are non-invasive (Baron et al., 2015). In the present study, hydration% derived from BIA was only correlated with Usg. BIA is frequently used to measure bodily water compartments, such as TBW, ECW and ICW (Bosy-Westphal, Schautz, Later, Kehayias, Gallagher, & Müller, 2013). However, it has not been used widely to measure hydration status. A prior study from CeSSIAM showed that hydration measurements by BIA from 40 lactating women in the region of Quetzaltenango, Guatemala, did not differ significantly from urine osmolality measurements (Maldonado et al., 2018). However, no other studies on hydration status measured by BIA are available. Therefore, BIA cannot be considered a validated tool to measure hydration status.

Previous studies have shown that body water compartments were associated with weight and/or height (Aloia, Vaswani, Flaster, & Ma, 1998). Findings from the current study also showed significant correlations between TBW and ECW with Height and Weight. However, no significant correlation with hydration status (all four methods) was found. In contrast, significant correlations were found for hydration status from BIA with BMI and Fat Mass, in which women with a higher BMI and more fat mass had a better hydration status. This could be due to the fact that these are non-dependent data that were based on the same measurements and formulas. Although, it is in accordance with the findings from Sartorio et al. (2005), who found that obese women had a significantly higher TBW and ECW than non-obese women and thus a better hydration status (Sartorio et al., 2005). However, this could not be confirmed based on the weak and non-significant correlation between Uosm and BMI and Uosm and Fat Mass in the current study.

Furthermore, the present study is subjected to a few limitations. The first limitation of this study is that the age of the baby was not considered nor collected. Infants of different ages demand different amounts of breastmilk and can be partially or exclusively breastfed. This may have had influence on the hydration status of the mother (Martinez, 2014). Therefore, prior studies mostly chose to investigate infants up to 6 months old, since those are often exclusively breastfed (Rolker et al., 2016). For this study it was chosen to include lactating women in any stage of lactation to reach a sufficient number (40) of lactating participants. Secondly, an equal number of Q'eqchi' Mayan, Garifuna and Ladina women was desired to be included in the study. However, most women visiting the CAIMI Health Centre in Livingston were Q'eqchi' Mayan, therefore it was not possible to include a similar number of women from every cultural origin in the study. Also, cultural origin was determined by appearance and wearing or not of traditional clothing, since asking for cultural origin might have been too much of a sensitive topic. Especially women considered Ladina might have been Q'eqchi' Mayan. Thirdly, no BIA could be done for the control group, because of a lack of electrodes, therefore less descriptive data were collected for this group. Fourthly, spot urine instead of 24-h urine was collected to measure Ucol, Usg and Uosm. Collections of 24-h urine are widely accepted to use for measurements of osmolality and specific gravity (Bottin et al., 2016). However, 24-h urine collections are inconvenient for individual hydration measurements. A study by Bottin et al. (2016) showed that afternoon and early-evening spot urine osmolality and specific gravity were equivalent to 24-h urine concentrations in healthy young adults and may therefore be a convenient alternative to 24-h urine samples to determine daily hydration. The study stated as well that (first-)morning samples and late-evening samples overestimate the Uosm concentrations (Bottin et al., 2016). In the current study morning or early-afternoon samples were used, therefore the outcomes may have been an overestimation of the true hydration status. However, first morning samples were most likely not used. Lastly, no standard cut-off values

exist for the methods of measuring hydration status. This could explain the non-optimal sensitivity and specificity of Ucol, Usg and BIA. After literature research, the cut-off values used in the current study were established (table 1)(Armstrong et al., 1998, 2012, 2005; Bosy-Westphal et al., 2013; Rosinger, 2015b; Soto-Méndez et al., 2016). However, universally accepted cut-off values should be established, since it determines whether someone is euhydrated or hypohydrated.

Some strengths of this study were that a reasonable sample size of 80 women in total was included and that data of lactating women were compared with a control group. Moreover, the fact that four different methods were used to determine hydration status and that three of those were correlated was another strength of the study.

Recommendations for future research about hydration would be to include fluid intake, in this way hydration status and fluid intake can be compared. Moreover, because of the high numbers of hypohydration in Livingston, research on drinking water access and water quality in this region would be useful and important, since little water access and low water quality can increase the risk of dehydration (Rosinger, 2015b, 2015a). Own observations led me to the conclusion that clean drinking water is not guaranteed in the region of Livingston.

Conclusion

In conclusion, it can be stated that hypohydration is a problem in lactating women, but also in non-lactating women, on the Caribbean coast of Guatemala. Explanations for this problem could be the extra loss of fluid during lactation, the tropical climate in this region and too little water consumption. Moreover, from the correlation between Ucol, Usg and Uosm it can be concluded that these are viable methods to establish hydration status. Additionally, inexpensive and user-friendly approaches like Ucol and Usg measurements could be introduced in the local health center for assessment of hydration status. Also, campaigns about hydration during lactation could be helpful to tackle the problem of dehydration in the region of Livingston, Guatemala. More research on the use of BIA to establish hydration status should be done. However, inexpensive and non-invasive validated methods to establish hydration status are already available.

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Appendix I

Table 6. Multiple comparisons ANOVA, descriptive data LG.

Dependent Variable	(I) Origin	(J) Origin	Mean Difference (I-J)	p-value
Age (years)	Garifuna	Ladina	-1.375	0.891
		Q'eqchi' Maya	-2.083	0.674
	Ladina	Garifuna	1.375	0.891
		Q'eqchi' Maya	-.708	0.955
Weight (Kg)	Garifuna	Ladina	2.056	0.959
		Q'eqchi' Maya	11.881	0.138
	Ladina	Garifuna	-2.056	0.959
		Q'eqchi' Maya	9.825	0.252
Height (cm)	Garifuna	Ladina	12.175*	0.000
		Q'eqchi' Maya	13.671*	0.000
	Ladina	Garifuna	-12.175*	0.000
		Q'eqchi' Maya	1.496	0.789
BMI (Kg/m2)	Garifuna	Ladina	-2.930	0.647
		Q'eqchi' Maya	0.975	0.929
	Ladina	Garifuna	2.930	0.647
		Q'eqchi' Maya	3.905	0.321
Waist Circumference (cm)	Garifuna	Ladina	1.000	0.987
		Q'eqchi' Maya	6.958	0.385
	Ladina	Garifuna	-1.000	0.987
		Q'eqchi' Maya	5.958	0.494

**significant difference, $\alpha<0.05$*

Appendix II

Table 7. Differences in hydration status between LG and CG for Ucol, Usg and Uosm, separately for cultural origin. Done by a Mann-Whitney U test.

	Ucol (1-8)	**Usg (g/mL)**	**Uosm (mOsm/kg)**
LG Q'eqchi' Maya (n=24) median [25th;75th percentile]	4 [2.25;6]	1.023 [1.017;1.026]	679.5 [426;826]
CG Q'eqchi' Maya (n=14) median [25th;75th percentile]	5 [2.75;6]	1.021 [1.012;1.024	413 [269.25; 615.25]
U	154.5	137.5	91.5
p-value	0.672	0.355	0.021*
LG Garifuna (n=8) median [25th;75th percentile]	5 [4;6]	1.026 [1.019;1.032]	841.5 [508;1463]
CG Garifuna (n=10) median [25th;75th percentile]	4 [3;6]	1.017 [1.008;1.022]	427.5 [208.75;554.75]
U	26.5	15	12
p-value	0.221	0.026*	0.013*
LG Ladina (n=8) median [25th;75th percentile]	5 [4;6]	1.026 [1.022;1.030]	702 [648.25;1312.5]
CG Ladina (n=16) median [25th;75th percentile]	4 [3;6]	1.024 [1.017;1.027]	702 [471.74;860.5]
U	50	46.5	49
p-value	0.372	0.282	0.358

**significant difference, α<0.05*

Appendix III

Table 8. Mean differences and p-values ANOVA for differences in hydration status between cultural origin groups.

		Q'eqchi' Maya		**Garifuna**	
		Mean difference	*p-value*	*Mean difference*	*p-value*
Ladina	*Ucol*	0.667	0.592	0.125	0.988
	Usg	0.004	0.439	0.001	0.975
	Uosm	253.792	0.263	47.875	0.968
	BIA	2.476	0.688	2.968	0.699
Garifuna	*Ucol*	0.792	0.480		
	Usg	0.003	0.597		
	Uosm	301.667	0.156		
	BIA	5.444	0.177		

Appendix IV

Table 9. Correlation between body composition and hydration status by Spearman's rank-order correlation test.

		Ucol	Usg	Uosm	BIA
Height	*Spearman's rho*	-0.124	0.064	0.248	-0.183
	p-value	0.447	0.693	0.124	0.253
Weight	*Spearman's rho*	-0.047	-0.201	-0.072	0.291
	p-value	0.775	0.213	0.659	0.065
BMI	*Spearman's rho*	-0.045	-0.281	-0.237	0.437**
	p-value	0.781	0.079	0.142	0.004
Fat Mass	*Spearman's rho*	-0.030	-0.146	-0.075	0.361*
	p-value	0.853	0.368	0.645	0.020
Fat Free Mass	*Spearman's rho*	-0.044	-0.147	0.026	0.065
	p-value	0.786	0.365	0.873	0.687
Visceral Fat	*Spearman's rho*	0.000	0.070	0.095	0.065
	p-value	0.998	0.667	0.560	0.686

**Correlation is significant at the 0.05 level (2-tailed)*
***Correlation is significant at the 0.01 level (2-tailed)*

Table 10. Correlation between TBW and ECW with height and weight.

		Height	Weight
TBW	*Spearman's rho*	0.578**	0.761**
	p-value	0.000	0.000
ECW	*Spearman's rho*	0.486**	0.781**
	p-value	0.001	0.000

***Correlation is significant at the 0.01 level (2-tailed)*